

**MIP/QCM SENSORS FOR HIGH SENSITIVITY-FAST SENSING OF SMALL
MOLECULES IN SOLUTION**

FIELD OF THE INVENTION

This invention relates to the field of molecularly imprinted polymers (MIPs) and,
5 in particular, to their use in quartz crystal microbalance (QCM) sensor devices.

BACKGROUND OF THE INVENTION

Designing sensors for pollutants has long been, and still is, an important technological challenge. U.S. Patent No. 5,990,684, issued to Merrill (the '684 patent), which patent is herein incorporated by reference in its entirety, is directed to a "method and apparatus for continuously monitoring an aqueous flow to detect and quantify ions." The method involves providing a conduit having at least one ion collection portion, disposing the aqueous flow through the conduit, attracting target ions to the ion collection portion such that they are bonded to the ion collection portion, and detecting a contaminant, or contaminants, based upon a predetermined property of the plurality of target ions bonded to the ion collection portion. In the preferred embodiment of the method, the predetermined property is a conductivity of the target ions, and the detecting step involves measuring a change in conductivity of the collection portion as ions are bonded and comparing that conductivity to a predetermined conductivity. The apparatus includes a conduit into which an ion collection portion is disposed, a sensor that senses ions collected on the ion collection portion and sends a signal corresponding to a value of a predetermined property of the ions, and a microprocessor in communication with the sensor and programmed to process the signal and determine the presence of the at least one contaminant based upon the processed signal

A particularly useful molecular sensing technology is based on quartz crystal microbalances (QCMs), a family of very sensitive and inexpensive sensors that produce a frequency shift in quartz crystal vibration when molecules interact with the sensor surface. These sensors can be used in both the gas and liquid phases, and generate a 5 signal that is generally proportional to the concentration of the interacting molecules.

Gas phase QCMs are commercially used in the electronic industry.

In the liquid phase, QCMs have been coupled to antibodies, allowing for the selective sensing of the antibody conjugate. More recently, a technique called "molecular imprinting" has also been used. Molecular imprinting polymerization (MIP) is a 10 molecular technology that allows for the selective recognition of targeted molecules by cross-linked polymers. This technique consists of coating a cross-linked templated polymeric layer to the QCM surface.

Though recent, the molecular imprinting technique is known in the art. For example, U.S. Patent No. 5,630,978 discloses a method for preparing mimics of a wide 15 variety of drugs and other biologically active molecules using molecular imprinting techniques. Additionally, U.S. Patent No. 5,959,050 is directed to a molecularly imprinted support formed from at least two distinct acrylic monomers and at least one imprinted molecule. What is needed in the art, therefore, is the use of MIP techniques in QCM-based sensors for continuous on-line monitoring of harmful, water-borne organic 20 contaminants.

SUMMARY OF THE INVENTION

The present invention combines MIP molecular recognition technology, which allows for the selective recognition of targeted molecules by cross-linked polymers, with

the sensitivity offered by QCM sensors. The MIP/QCM sensors provide high sensitivity and fast sensing for small molecules in a solution, such as aqueous organic pollutants. This allows for continuous on-line monitoring of contaminants.

The process of combining MIP techniques with QCMs includes choosing the 5 proper monomers and on-chip polymerization of imprinting agents to provide MIP-functionalized QCM probes. In experimentation with the present invention, a MIP/QCM sensor has been fabricated that can recognize hexachlorobenzene with an excellent response time and at concentrations consistent with applications in pollution control.

Therefore, it is an aspect of this invention to apply MIP molecular recognition 10 technology to QCM sensors.

It is another aspect of the invention to provide on-chip polymerization of imprinting agents to provide MIP/QCM sensors.

It is a further aspect of the invention to provide continuous on-line monitoring for specified contaminates.

15 It is a further aspect of the invention to provide MIP/QCM sensors that are efficient and sensitive to detecting specified pollutants.

These aspects of the invention are not meant to be exclusive and other features, aspects, and advantages of the present invention will be readily apparent to those of ordinary skill in the art when read in conjunction with the appended claims.

20

DETAILED DESCRIPTION OF THE INVENTION

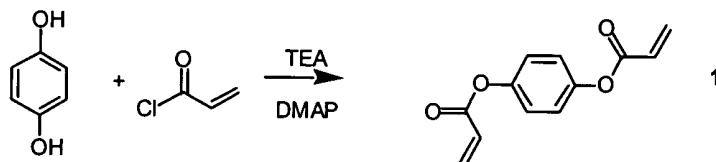
As disclosed in the incorporated '684 patent at column 4, lines 57-65, an apparatus for the continuous monitoring of contaminants in solution includes a conduit into which an ion collection portion is disposed, a sensor that senses ions collected on the ion

collection portion and sends a signal corresponding to a value of a predetermined property of the ions, and a microprocessor in communication with the sensor and programmed to process the signal and determine the presence of the at least one contaminant based upon the processed signal.

5 In preferred embodiments of the present invention, QCM sensors are used to detect the collected molecules and ions. Said molecules are attracted to the QCMs by molecularly imprinted polymers programmed to recognize the specific molecules. The collection and recognition of these molecules is then translated into a signal that a microprocessor interprets as the presence of specific contaminants.

10 The first phase of the invention is monomer choice and synthesis. Several acrylic monomers containing aromatic linkers were synthesized under the assumption that the aromatic groups, in particular the electron-rich groups, would induce favorable electronic interactions. These π -interactions are the only directional attractive forces that can be used for molecules like chlorinated aromatics or polycyclic aromatic molecules.

15 The structures of six monomers (1-2, 4-6, and 8) synthesized for this invention are indicated in the following figures along with the synthetic schemes used to obtain them. Detailed experimental procedures follow.



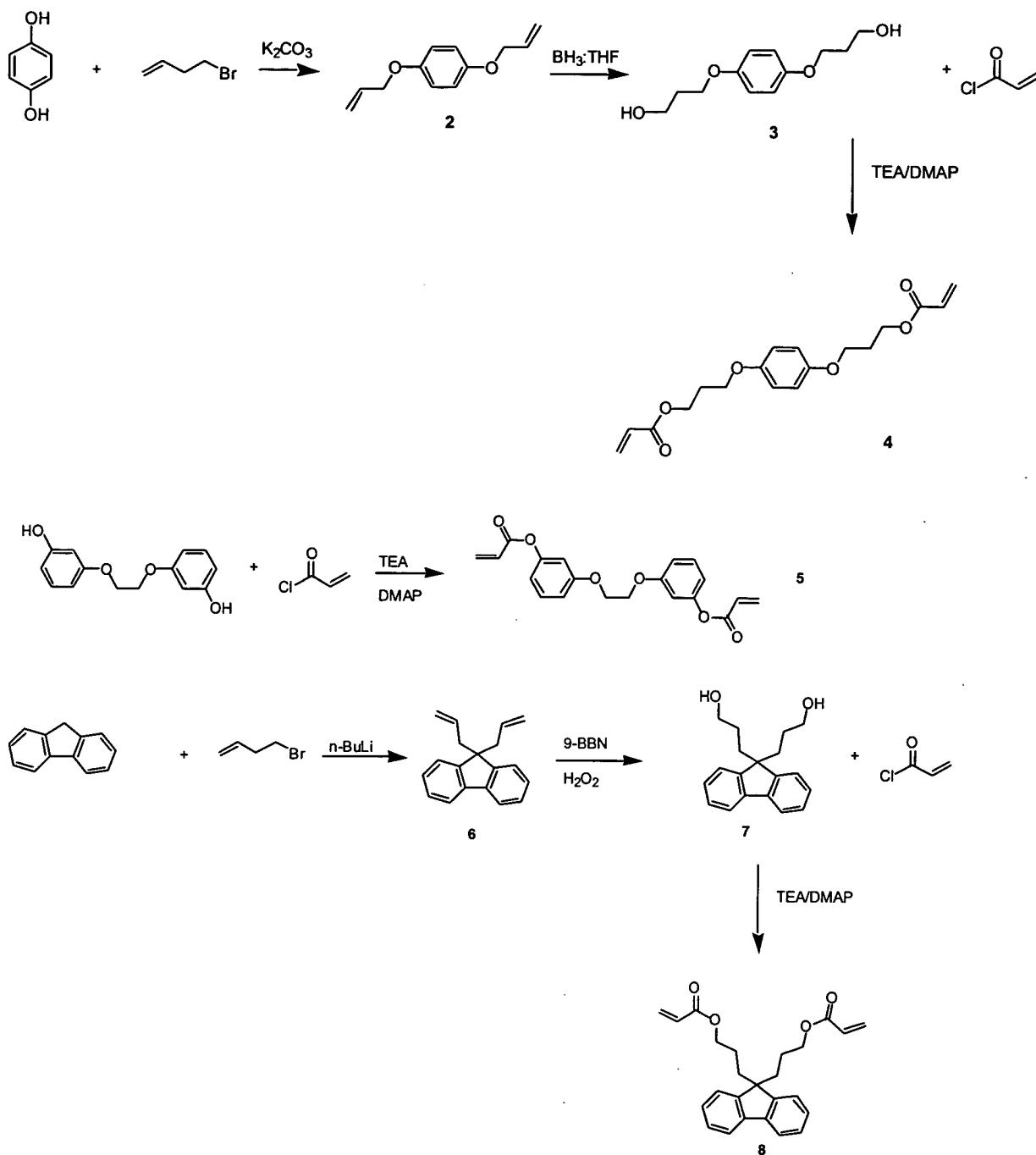


Figure 1. Synthetic routes for acrylic monomers

All reactions were carried out in oven-dried glassware under Argon unless otherwise indicated. Tetrahydrofuran and dichloromethane were distilled over $Na/benzophenone$ and CaH_2 respectively prior to use. All 1H NMR was measured at 200

MHz in CDCl_3 . 9,9 diallyl fluorene was prepared according to Waymouth et al. *J. Am. Chem. Soc.*, 1994, 116(5), 1845-54.

Preparation of (1)

To a solution of hydroquinone (1.0 g, 9.1 mmol), TEA (1.6 mL, 2.0 mmol), and 5 DMAP (cat.) in CH_2Cl_2 (50 mL), acrolyl chloride (2.8 mL, 2.0 mmol) was added slowly and stirred overnight. The reaction mixture was then washed with brine (3 x 30 mL), the organic layer was collected, dried over MgSO_4 , and passed through a short path of silica to remove any unreacted hydroquinone. Solvent was removed by rotary evaporation to give 1.45 g (73%) product. ^1H NMR (δ , ppm) 5.9 (CH, 1 H, d, $J=15$ Hz), 6.5 (CH, 1H, s), 6.8 (CH, 1H, s), 7.1 (Ar, 2 H, s).

Preparation of (2), diallyl hydroquinone

A solution of hydroquinone (500 mg, 4.5 mmol) in acetone (20 mL) was degassed with Ar for 10 minutes. K_2CO_3 (1.88 g, 13.6 mmol) and allyl bromide (1.17 mL, 13.6 mmol) were added and the solution was refluxed overnight. The brown solution was then 15 cooled and filtered. Acetone was removed by rotary evaporation. The resulting oil was purified by flash column chromatography (2:1 hexane/ethyl acetate) to afford a white solid (1.0g, 89%). ^1H NMR (δ , ppm) 1.4 (t, $J= 6$ Hz), 2.0 (CH₂, q, $J= 9$ Hz), 4.8 (CH₂, q, $J= 9$ Hz), 5.3 (CH₂, $J= 10$ Hz).

Preparation of (3)

20 (2) (500 mg, 2.6 mmol) was dissolved in THF (10 mL) and cooled in a dry ice bath. $\text{BH}_3\text{:THF}$ (1M solution, 5.26 mL) was added drop wise over 15 minutes. The solution was allowed to warm to room temperature and stirred for three hours. A 1:1

mixture of 3M NaOH and 30% H₂O₂ (10 mL) was added and the solution was stirred for two days. The milky white solution was washed with brine (3 x 10 mL) and purified by flash column chromatography (1:3 hexane/ethyl acetate) to afford a white powder (503 mg, 85%). ¹H NMR (δ , ppm) 1.8 ppm (OH, t, J = 6 Hz), 2.0 (CH₂, q, J = 9 Hz), 3.8 (CH₂, q, J = 9 Hz), 4.3 (CH₂, q, J = 10 Hz), 6.8 (Ar, s).

Preparation of (4)

To a solution of (3) (50 mg, 0.2 mmol) and TEA (0.06 mL, 4 mmol) in CH₂Cl₂ (10 mL), acryloyl chloride (0.04 mL, 0.4 mmol) was added and stirred overnight. The reaction mixture was washed with brine (3 x 10 mL). The organic layer was collected and dried over MgSO₄. The solvent was removed by rotary evaporation and the resulting oil purified by flash column chromatography (5:1 (v:v) hexane/ethyl acetate) to give a white crystalline powder (43 mg, 64%). ¹H NMR (δ , ppm) 1.8 ppm (OH, t, J = 6 Hz), 2.0 (CH₂, q, J = 9 Hz), 3.8 (CH₂, q, J = 9 Hz), 4.3 (CH₂, q, J = 10 Hz), 6.8 (Ar, s).

Preparation of (5) and (8)

This esterification was done as in the preparation of (1). Yields: 87% and 68 %, respectively. ¹H NMR (**5**) (δ , ppm) 4.1 (t, 4H CH₂O, J =22 Hz), 5.9 (CH, 1 H, d, J = 15 Hz), 6.5 (CH, 1H, s), 6.8 (CH, 1H, s), 7.1 (Ar, m, 2H), 7.8 (Ar, m, 1H).

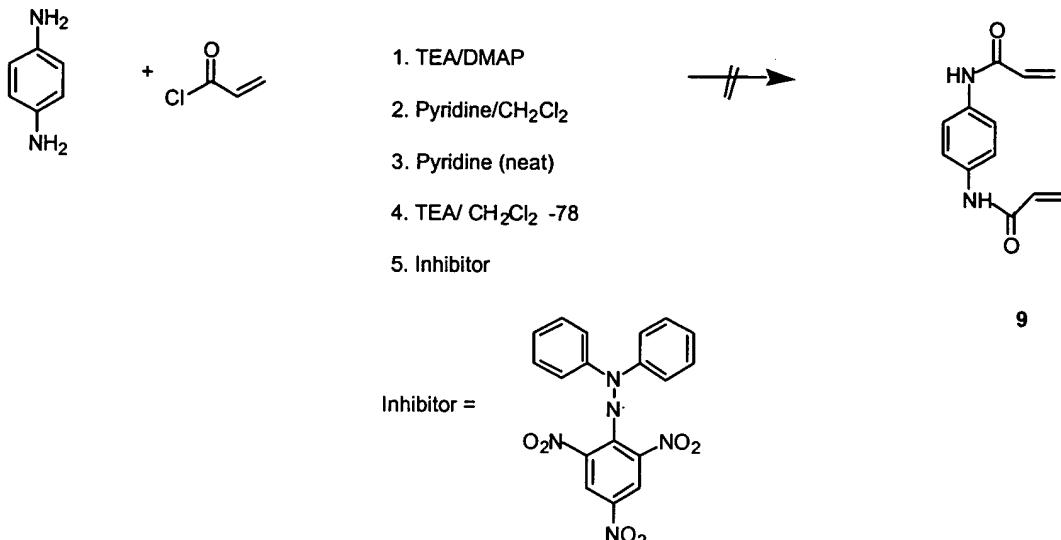
Preparation of (6)

This was synthesized according to literature procedures, Knight, K. S., Wang, D., Waymouth, R. M., Ziller, J. *J. Am. Chem. Soc.*, **1994**, *116*, 1845-54.

Preparation of (7)

Hydroboration procedure was identical to that of (3). Yield: 81%.

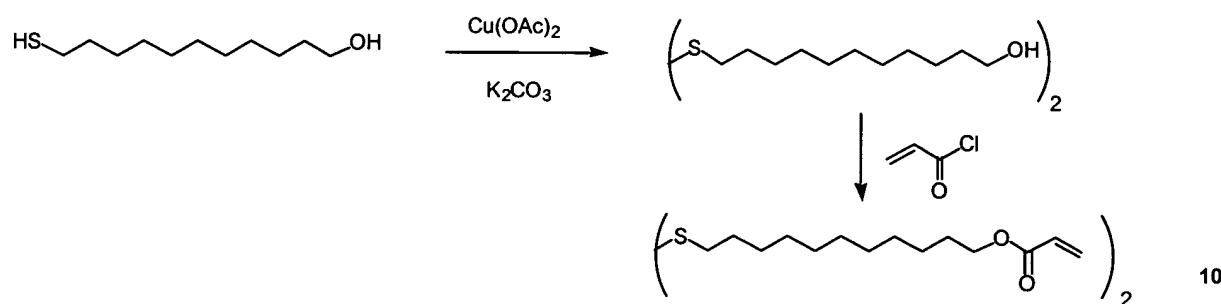
Repeated attempts to synthesize bisacrylamide (9) proved unsuccessful. The five conditions used to synthesize (9) from 1,4-phenylenediamine and acryloyl chloride are outlined in figure 2.



5

Figure 2. Attempts to synthesize bisacrylamide.

Shown in figure 3, the acrylic-capped n-alkanethiol (10) was also synthesized as a monomer able to modify the gold surface by making so-called "self-assembling monolayers" (SAMs).



10

Figure 3. Acrylic-capped n-alkanethiol.

Once proper monomers have been chosen, the next phase includes on-chip polymerization of imprinting agents to provide MIP-functionalized QCM probes.

Several approaches were explored for the synthesis of heavily cross-linked acrylic networks on the gold-made surface of a QCM probe.

The on-chip cross-linked coatings synthesized and studied for this invention were all obtained by fast UV-photopolymerization of a thin liquid film containing a

5 commercial bis- or trisacrylate (tripropylene glycol diacrylate (TPGDA) or trimethylopropane triacrylate (TMPTA)), a reactive diluent (benzyl methacrylate), a photoinitiator (2-benzyl-2-dimethylamino-4'-morpholino-butyrophenone (Irgacure 369), azo-bis(isobutyronitrile (AIBN) or tetraethylthiuram disulfide (TETD)) and various additives aimed at improving the adhesion of the final cross-linked polymer on the gold

10 surface. The initial thin liquid film was obtained by spin coating a few microliters of a solution containing the reagents diluted in tetrahydrofuran (THF) or dichloromethane. The film had previously been deposited on the gold surface of the chip. The light source used in these experiments has a very high intensity allowing most of the

15 photopolymerization to occur in less than 20 seconds. The exact amounts of each monomer/reagent and detailed experimental conditions are provided in the annex.

Preliminary experiments with 'simple' acrylic networks obtained by photoirradiation of TPGDA and benzyl methacrylate indicated a very poor adhesion of the film on the metallic surface: the film peeled off instantaneously after immersion in either dichloromethane or acetonitrile. This behavior was both expected and surprising.

20 Surprising because claims made in the recent literature imply that conditions can be found to obtain good adhesion of acrylic or styrenic polymers on a gold surface. Expected as this problem has a long history in polymer science and engineering and is still considered today as one of the key problem in UV-curing on metallic surfaces (UV-

cured paintings or plastic coatings on cars are traditional examples). The physics of the phenomenon is complex in its details, but the rationale for the poor adhesion is well known and understood: the polymer film shrinks during the curing while the underlying metallic substrate does not, generating a very large amount of stress at the interface.

5

Strategies that were successively considered to solve this problem include:

10

1. use of acrylic acid as a co-monomer,
2. use of itaconic acid as a co-monomer,
3. modification of the gold surface with a thiol-capped acrylic monomer able to self-assemble on the surface,
4. use of tetraethylthiuram disulfide as a photoinitiator or chain-transfer agent,
5. use of triacrylate (TMPTA) as the multifunctional monomer,
6. use of bis(dehydroalanine) (11) (DBHA) as the multifunctional monomer.

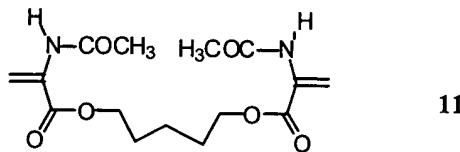


Figure 4. Bis(dehydroalanine).

15

The rationale for the above curing conditions is as follows:

(1 and 2). Introduce carboxylic acid for improved adhesion with the gold surface.

This strategy has been recently used in a MIP experiment (Haupt, K.; Noworyta, K.; Kuter, W. *Anal. Commun.*, 1999, 36, 391-393).

(3 and 4). Introduction of sulfur-containing groups for improved adhesion with the gold surface.

(5). Different stress buildup in the film.

(6). Monomer was available and had been shown to improve adhesion on several substrates.

The coatings obtained using strategies 1-5 showed adhesion properties. However, the use of monomer (11) is the preferred method, especially in adhering to metal surfaces.

Initial experiments on the acrylic network showed that enough adhesion can be obtained to remove the template (hexachlorobenzene) from the imprinted matrix. After removal of the template, QCM measurements were performed with aqueous solutions of hexachlorobenzene (1.3 to 2.8×10^{-8} M), benzene (5×10^{-8} M), cyclohexane (5×10^{-8} M), chlorobenzene (5×10^{-8} M) and anisole (5×10^{-8} M). The results are reproduced in the following figure:

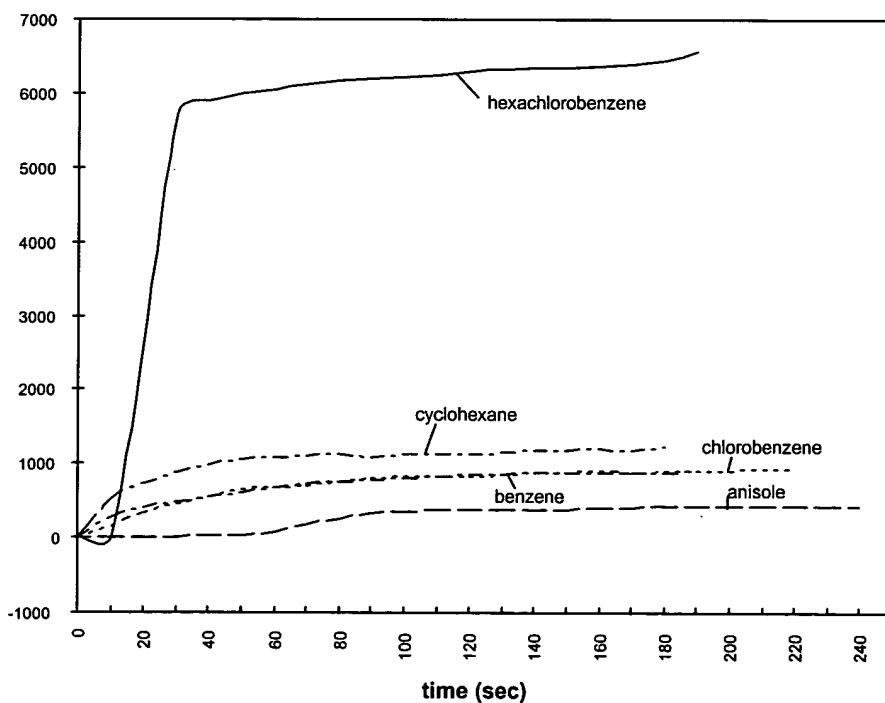


Figure 5. QCM measurements in aqueous solutions.

The results can be summarized as follows. The sensor displays selectivity with regard to cyclohexane, benzene, chlorobenzene and anisole. A signal at least six times

stronger is obtained for hexachlorobenzene than for the other molecules. The response time is excellent; a stable signal is obtained after less than 20-30 seconds. A MIP/QCM sensor based on a non-imprinted polymer coating (fabricated in the absence of hexachlorobenzene as a template) did not display any signal. Preliminary experiments 5 demonstrated that the designed sensor works as efficiently under acidic (pH=5), neutral (pH=7), and basic conditions (pH=9).

Although the present invention has been described with reference to certain preferred embodiments thereof, other versions are readily apparent to those of ordinary skill in the art. Therefore, the spirit and scope of the appended claims should not be 10 limited to the description of the preferred embodiments contained herein.